



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of: Hogarth et al
Serial No:
Filed: 31 August 2003
For: Polypeptides with Fc binding ability

RULE 132 DECLARATION

Hon. Commissioner of Patents and Trademarks

Washington, DC 2021

SIR:

I, Peter Hudson of 36 Fuchsia Street, Blackburn, VIC, Australia 3130, do solemnly and sincerely declare as follows:

1. I am currently employed as the Program Leader of Diagnostic and Therapeutic Technologies with the division of Health Sciences and Nutrition of the Commonwealth Scientific and Industrial Research Organisation (CSIRO). I also hold the position of Scientific Director of the Co-operative Research Centre for Diagnostics and Therapeutic Technologies and am also an Adjunct Professor of the Department of Biochemistry at LaTrobe University, Victoria, Australia.
2. My educational background includes a BSc (Hons) in Biochemistry from the University of Adelaide (1975), a PhD from the University of Cambridge, UK (1979) and an Adjunct Professorship in Biochemistry at LaTrobe University (1993). I have been elected a Fellow of the Australian Academy of Technological Sciences and Engineering (FTSE, 2002), awarded the prestigious AMRAD-Pharmacia Biotechnology Medal from the Australian Society for Biochemistry and Molecular Biology for 'distinguished contributions to the field of molecular biology' in 1997, awarded the CSIRO Chairman's Medal (1997) for the development of a successful veterinary vaccine and awarded the Boehringer Medal for Biochemistry (1987) for the most outstanding under 36-year old scientist in Australian Biochemistry. I have been the author or co-author of over 100 publications.
3. My research experience has been focussed on protein structure and engineering, including the development of protein-based diagnostic tests and the exploitation of many gene cloning and expression technologies. At CSIRO from 1985 to 1994, I was involved in the development of a protein-based vaccine to IBD-virus and designed and developed protein display technologies which included the use of spacer polypeptides and spacer proteins. Since 1992, I have led a Program for Protein Engineering and developed generic antibody designs and novel display technologies, many using spacer molecules. Recent achievements have included the design and production of novel high-avidity antibody multimers and immunotoxins, both including the use of spacer polypeptides.

4. I am not employed by the assignee of the present US patent application and have no pecuniary interest in the subject invention.
5. I understand that the priority date of the present US patent application is 16 September 1994.
6. I have reviewed the Advisory Action dated 11 March 2003 issued in respect of the parent patent application, US application No. 09/633,147, and the rejection relating to the use of the term "spacer" in claim 77. The Examiner considers that the specification does not define what the "metes and bounds" are for a spacer.
7. I understand that claim 77 reads as follows:

77. A method for testing a compound for its ability to act as an antagonist of Fc receptor comprising:

 - (1) producing a recombinant soluble polypeptide with Fc binding ability, wherein said recombinant soluble polypeptide comprises
 - (a) an Ig binding domain of said Fc receptor or a fragment thereof, and
 - (b) a spacer for spacing said recombinant soluble polypeptide from a solid surface,
 - (2) contacting said compound with said recombinant soluble polypeptide,
 - (3) contacting a mixture of said compound and said recombinant soluble polypeptide with an immune complex,
 - (4) measuring the degree to which said compound inhibits binding of said immune complex to said recombinant soluble polypeptide in (3), and
 - (5) identifying the compound which inhibits binding of said recombinant soluble polypeptide with said immune complex as an antagonist of said Fc receptor.
8. The term "spacer" is a commonly used term in protein chemistry which refers to an entity used to distance a molecule or an antigen, such as a polypeptide, from another molecule or a surface on which an assay is to be performed, such as the polystyrene surface of an ELISA plate. A spacer may be any of a number of molecules but are most typically selected from streptavidin, biotin, a peptide, polypeptide, dextran, single protein domain or a larger protein complex such as an antibody.
9. I note that at page 58 lines 4 to 15 of the specification, it is stated that "using standard protocols for attachment of proteins to ELISA plates it is clear that "spacing" the protein with Fc binding ability away from the surface improves the activity of the protein". This passage also specifically states that an antibody, protein, dextran etc. may be used as a spacer. This indicates to me that, consistent with my understanding of what is encompassed by the term "spacer", any molecule may fulfil this role if it serves to distance the polypeptide with Fc binding ability from a solid surface. I note that the specification particularly exemplifies antibody spacers, namely antibodies 8.2 and 8.26 directed against

anti-human FcγRII (see page 58 lines 11-12 and 31-33), which had been previously described in a paper by Ierino, FL *et al.* An abstract of this paper is attached as exhibit PH10.

10. I also note that at page 18 lines 26 to 35 of the specification, there is a disclosure that the polypeptide with Fc binding ability or Fc receptor may be attached to a solid support in a manner which leaves the Fc binding site "free". This indicates to me that the Fc binding site is required to be accessible for binding of a ligand. An established issue with the binding of a molecule such as a polypeptide to a support is that the molecule, when attached to the support, may not present itself in a conformation that is conducive to the binding of a ligand.
11. An effect of a spacer is to allow the polypeptide "room" to adopt its native conformation and position the polypeptide at a sufficient distance from another molecule or a surface so as to avoid steric interference and thereby present any conformational epitope or ligand binding site in a manner such that it will be recognised by an antibody that recognises the conformational epitope or a ligand that binds to the ligand binding site.
12. I refer to the attached exhibits PH1 to PH9 which are a sample of abstracts of papers published prior to the priority date of the present application and which refer to the use of spacers for spacing a molecule from another molecule or solid surface. Among these abstracts, I note that the particular spacers mentioned include streptavidin (PH1), biotin (PH3), aminocaproyl groups (PH4) and fetuin (PH8). At the abovementioned priority date, it was well within the routine skill of persons skilled in the art to link two molecules, or link a molecule to a solid support, via a spacer and, further, it is routine to test whether the use of a particular spacer is successful or not (ie preserves a conformational epitope or ligand binding site and presents it in a manner whereby it is accessible). For instance, when in the past I have needed to make use of a spacer, I have routinely constructed genes that encode fusion proteins comprising a ligand binding site separated by a spacer polypeptide or spacer domain. These have included both the display of an antibody fragment on bacteriophage surfaces and the display of a conformational epitope on antibody fragments.
13. In the case of the display of an antibody fragment on bacteriophage surfaces, published in *Nucleic Acids Research* 19(15):4133-4137 (1991) and entitled 'Multi-subunit proteins on the surface of filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains' by Hoogenboom HR, Griffiths AD, Johnson KS, Chiswell DJ, Hudson P, and Winter G, it was demonstrated that the display of proteins on the surface of bacteriophage offers a powerful means of selecting for rare genes encoding proteins with binding activities. The work involved, in particular, that antibody heavy and light chain variable (V) domains fused as a single polypeptide chain to a minor coat protein of filamentous phage fd (ie a spacer). It was also demonstrated that heterodimeric Fab fragments can be assembled on the surface of bacteriophage by linking one chain to the phage coat protein (ie a spacer), and secreting the other into the bacterial periplasm, thereby allowing refolding to its native conformation and, subsequent availability for ligand binding. There was nothing involved in the production of the fusion proteins (ie including a spacer) which was other than routine and it was a simple process to determine whether or not the antibody fragments retained the ability to bind ligands.
14. In the case of the display of a conformational epitope on antibody fragments, published in *J Immunol Methods* 171(2):211-226 (1994) and entitled 'Recombinant single-chain antibody

peptide conjugates expressed in *Escherichia coli* for the rapid diagnosis of HIV' by Lilley GG, Dolezal O, Hillyard CJ, Bernard C, and Hudson PJ, it was demonstrated that recombinant single chain Fv (scFv) antibody fragments could form the basis of a rapid, whole-blood diagnostic assay. The scFv described in this study was derived from a monoclonal antibody having a high affinity for glycophorin A which is an abundant glycoprotein on the human red blood cell membrane surface. The prototype reagent built around the scFv was designed to detect, in whole-blood samples, the presence of antibodies that have arisen through infection with a foreign organism such as human immunodeficiency virus (HIV). The scFv was comprised of an antibody heavy-chain variable domain (Vh) joined by a 15 amino acid spacer (GGGGS-GGGGS-GGGGS) to a light-chain variable domain (Vl) terminated by a C-terminal octapeptide spacer (FLAG peptide) and a 35 amino acid fragment from the gp41 surface glycoprotein of HIV-1. The scFv-epitope fusion protein retained anti-glycophorin activity which could be detected directly in culture supernatants by ELISA. Furthermore, the scFv-epitope fusion protein functioned efficiently in the whole-blood agglutination assay and was able to distinguish between HIV-1 positive and negative sera. Again, there was nothing involved in the production of the fusion protein which was other than routine and it was a simple process to determine whether or not the antibody fragments and the epitope retained their ability to bind ligands.

15. I therefore consider that at the priority date and at all times since, the term "spacer" as used in claim 77 would have been immediately and well understood by persons skilled in the art.

I declare that all statements made herein of my own knowledge and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 USC Section 1001 and that such wilful false statements may jeopardise the validity of the application or any patent issued thereon.

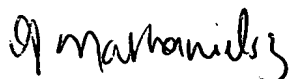
Signature:


 Professor Peter Hudson

Date:

October 10th 2003

Before:


 Anne Narvanielsz.

Appendix

- PH1. Peterman JH et al 1988, *J Immunol Methods*, 111(2):271-5. The immunochemistry of sandwich-ELISAs. IV. The antigen capture capacity of antibody covalently attached to bromoacetyl surface-functionalized polystyrene.
- PH2. Lapinjoki SP et al 1986, *J Immunoassay*, 7(1-2):113-28. An enzyme-linked immunosorbent assay for the antineoplastic agent vincristine.
- PH3. von Grunigen et al 1991 *Biol Chem Hoppe Seyler*, 372(3):163-72. Enzyme immunoassay with captured hapten. A sensitive gastrin assay with biotinyl-gastrin derivatives.
- PH4. Scott D et al 1984, *Mol Immunol*, 21(11):1055-60. Immunogenicity of biotinylated hapten-avidin complexes.
- PH5. Mage MG et al 1992, *Proc Natl Acad Sci USA*, 89:10658-10662. A recombinant, soluble, single-chain class I major histocompatibility complex molecule with biological activity.
- PH6. Bradshaw CG et al 1994, *J Med Chem*, 37(13):1991-5. Synthesis and characterization of selective fluorescent ligands for the neurokinin NK2 receptor.
- PH7. Piergentili A et al 1994, *Farmaco*, 49(2):83-7. Synthesis and muscarinic receptors affinity of a series of antagonist bivalent ligands.
- PH8. Zackrisson G et al 1986, *Acta Pathol Microbiol Scand*, 94(6):227-31. An enzyme-linked-immunosorbent assay method for detection of immunoglobulins to pertussis toxin.
- PH9. Erhard MH et al 1989, *Arch Toxicol* 63(6):462-8, Development of an ELISA for detection of an organophosphorus compound using monoclonal antibodies.
- PH10. Ierino FL et al 1993, *J Immunol*, 150(5):1794-803, Mapping epitopes of human Fc gamma RII (CDw32) with monoclonal antibodies and recombinant receptors.